FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005-3315

202 • 408 • 4000
FACSIMILE 202 • 408 • 4400

WRITER'S DIRECT DIAL NUMBER:

(202) 408-4079

ATLANTA
404•653•6400
PALO ALTO
650•849•6600

TOKYO
011•813•3431•6943
BRUSSELS
011•322•646•0353

May 26, 2000

ATTORNEY DOCKET NO. 03804.0114-02
CUSTOMER NO. 22852

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Re: Continuation U.S. Patent Application for Pharmaceutical Compositions and Utilization Thereof Particularly for the Treatment of Neurodegenerative Disorders
Inventors: Jacques Mallet, Frédéri Revah, and Jean-Marie Stutzmann

Sir:

We enclose the following papers for filing in the United States Patent and Trademark Office in connection with the above-referenced patent application:

1. a complete copy of the prior application including the oath or Declaration and drawings, if any, as originally filed. I hereby verify that the attached papers are a true copy of prior application Serial No. 08/624,469 as originally filed on May 10, 1996;

2. a Preliminary Amendment; and

3. a copy of the associate power of attorney in the prior application.

Applicants claim the right to priority under 35 U.S.C. § 120 to U.S. application Serial No. 08/624,469 filed May 10, 1996, which is incorporated by reference in the present application, and the right to priority under 35 U.S.C. § 119(a) to French application Serial No. FR93-11774 filed October 4, 1993.

This application is being filed under the provisions of 37 C.F.R. § 1.53(f). Applicants await notification from the Patent and Trademark Office of the time set for paying the filing fee.

Please accord this application a serial number and filing date.

Please send all future correspondence and Office Actions to the following address:

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
1300 I Street, N.W.
Washington, D.C. 20005-3315

Other than the filing fee, the Commissioner is hereby authorized to charge any additional filing fees due and any other fees due under 37 C.F.R. § 1.16 or § 1.17 during the pendency of this application to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Steven P. O'Connor

Steven P. O'Connor
Reg. No. 41,225

SPO/WLS:lab
Enclosures

PATENT
Attorney Docket No. 03804.0114-02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jacques MALLET *et al.* Continuation of application Serial No.: 08/624,469 Filed: May 26, 2000 For: PHARMACEUTICAL COMPOSITIONS AND UTILIZATION THEREOF PARTICULARLY FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS	Prior Group Art Unit: 1632 Prior Examiner: J. Martin

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Before examining this application on the merits, please enter the following amendment.

In the Specification:

Please insert before the first line, the sentence:

--This is a continuation of application Serial No. 08/624,469, filed May 10, 1996, which is a 371 of PCT/FR94/01142, filed September 29, 1994, both of which are incorporated herein by reference.--

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

In the Claims:

Please cancel claims 1-15 without prejudice or disclaimer of the subject matter recited therein and kindly enter the following claims:

--16. A recombinant virus selected from the group consisting of adenovirus, adeno-associated virus and herpes virus, said recombinant virus comprising a nucleic acid selected from the group consisting of:

(a) nucleic acids encoding a mutated form of p53 which antagonizes wild-type p53-mediated neuronal cell degeneration *in vitro*;

(b) the site for binding of p53 to DNA; and

(c) nucleic acids encoding an antisense RNA which inhibits expression of p53.

17. A recombinant virus according to claim 16, wherein said virus is an adenovirus.

18. A recombinant virus according to claim 16, wherein the nucleic acid comprises SEQ ID No. 2 or an active variant thereof.

19. A recombinant virus according to claim 16, wherein said virus comprises two nucleic acids selected from the group consisting of:

(a) nucleic acids encoding a mutated form of p53 which antagonizes wild-type p53-mediated neuronal cell degeneration;

(b) the site for binding of p53 to DNA; and

(c) nucleic acids encoding an antisense RNA which inhibits expression of p53.

20. A recombinant virus according to claim 16, wherein said virus is a

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

replication defective virus.

21. A recombinant virus according to claim 16, wherein the nucleic acid encodes the p53Val135 mutated form of p53.

22. A method of inhibiting toxicity in cultured neuronal cells comprising administering to said cells a nucleic acid selected from the group consisting of:

(a) nucleic acids encoding a mutated form of p53 which antagonizes wild-type p53-mediated neuronal cell degeneration *in vitro*;

(b) the site for binding of p53 to DNA; and

(c) nucleic acids encoding an antisense RNA which inhibits expression of p53.

23. The method according to claim 22, wherein the nucleic acid is a p53 antisense oligonucleotide.

24. The method of claim 23, wherein said oligonucleotide has the sequence of SEQ ID No. 1.

25. The method according to claim 22, wherein the nucleic acid is within a vector.

26. The method according to claim 25, wherein the vector is a replication defective virus.

27. A method for identifying compounds which at least partially inhibit the activity of the p53 protein, comprising the steps of:

a) treating a culture of neuronal cells sensitive to glutamate-induced excitotoxcity with a compound so that said compound enters said neuronal cells;

b) adding an excitotoxic amount of glutamate to the culture medium of

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

said neuronal cells.

c) comparing the amount of excitotoxicity measured in said neuronal cells with the amount of excititoxcity measured in neuronal cells which were not treated with said compound.

28. The method according to claim 27, wherein the neuronal cells are embryonic rat cortical neurons.

29. The method according to claim 27, wherein the compound is a recombinant virus according to claim 16.

30. The method according to claim 27, wherein the compound is a p53 antisense oligonucleotide.

31. The method according to claim 27, wherein the compound is a recombinant virus according to claim 19.--

REMARKS

With entry of this Preliminary Amendment, claims 16-31 will be pending in this application. Support for claims 16-26 may be found in the specification and in cancelled claims 11-22. Support for claims 27-31 may be found in the specification, for instance, at Example 2, which appears on pages 16 and 17.

Applicants do not believe that entry of this Preliminary Amendment requires payment of a fee, or an extension of time. If necessary, however, please grant any

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

extensions of time required to enter this Amendment and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

By: _____
Steven P. O'Connor
Reg. No. 41,225

Dated: May 26, 2000

LAW OFFICES
FINNEGAN, HENDERSON, FARABOW, GARRETT, & DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

Jacques Mallet, Frédéric Revah & Jean-Marie Stutzmann

PHARMACEUTICAL COMPOSITIONS AND UTILIZATION THEREOF PARTICULARLY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

U.S. National Stage of PCT/FR94/01142

English Translation of the International Application
(23 pages text; 1 sheet drawings)

Atty. Docket No. ST93049-US

CERTIFICATE OF MAILING (37 CFR § 1.10)

GB840707802 US
"Express Mail" Mailing Number

04 April 1996
Date of Deposit

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Assistant Commissioner for Patents, Washington, DC 20231, Attn. EO/US.

Julie K. Smith
(Type or print name of person mailing paper)

[signature]
(Signature of person mailing paper)

PHARMACEUTICAL COMPOSITIONS AND UTILIZATION THEREOF PARTICULARLY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

The present invention relates to pharmaceutical compositions and their use, especially in the treatment of neurodegenerative diseases. It relates more particularly to the use of compounds acting on the p53 protein or on its gene for the preparation of a pharmaceutical composition intended for the treatment of neurodegenerative diseases.

The p53 gene encodes a nuclear protein of 53 kDa. The wild-type gene encoding the native p53 has an antioncogenic activity [for a review, see for example Oren, FASEB J. 6 (1992) 3169]. In particular, the wild-type p53 protein is capable of inhibiting the formation of transformation foci in the fibroblasts of rodents infected with various combinations of oncogenes. The form mutated by deletion and/or mutation of this gene is on the contrary involved in the development of most human cancers [Baker et al., Science 244 (1989) 217]. Its mutated forms are also capable of cooperating with the ras oncogenes to transform murine fibroblasts. For this reason, the p53 protein or its gene have been widely studied as targets for the treatment of cancers. Moreover, Chopp et al. (Biochem.Biophys.Res.Com 182 (1992) 1201) have described a p53 expression in ischaemic mouse brain.

However, nothing indicates in these results if this expression constitutes a cause of neurodegeneration, or a parallel phenomenon. Furthermore, no therapeutic approach is envisaged or suggested in this document.

The present invention partially results from the demonstration that the p53 protein constitutes a mediator of neuronal degeneration. It also results from the demonstration that the use of compounds capable of at least partially inhibiting the activity of p53 protein can make it possible to block the process of neuronal death.

In order to study the molecular mechanisms of neuronal degeneration, the Applicant has used, as a model, mice in which the expression of the p53 gene has been inactivated [Donehower et al., Nature 356 (1992) 215]. Irreversible focal ischaemia experiments were performed on these mice, and the volumes of infarct were compared with those observed in control wild-type mice (same strain, same sex, same age, same supplier). The results obtained showed a statistically significant decrease of 20 % in the volumes of infarct after ischaemia in mice not expressing the p53 gene (cf. examples). Furthermore, the Applicant has also demonstrated that the use of anti-p53 antisense makes it possible to reduce the glutamate-induced death on cortical cell cultures. These results demonstrate that the p53 protein plays a mediating role in neuronal degeneration, an observation which has never been

reported in the prior art, and that a control of the activity of this protein makes it possible to combat neuronal death. The p53 protein, its gene and all the factors capable of interacting with it therefore constitute new pharmacological targets in the treatment of neurodegenerative processes. The invention therefore consists, in part, in the use of compounds capable of at least partially blocking the activity of p53 for the treatment of neurodegenerative diseases.

A first subject of the present invention consists in the use of a compound which at least partially inhibits the activity of the p53 protein for the preparation of a pharmaceutical composition intended for the treatment and/or the prevention of neurodegenerative diseases.

The compounds which at least partially inhibit the activity of the p53 protein for the purposes of the present invention may be compounds which act (i) on the synthesis of p53, at the transcriptional, translational or post-translational levels, or (ii) on the binding of p53 to DNA.

Among the compounds which act on the synthesis of the p53 protein, there may be mentioned the antisense nucleotide sequences capable of reducing or of suppressing the expression of p53, at the transcriptional or translational level.

Such sequences may indeed be directed against the p53 mRNA and act on its translation into protein:

they may be oligonucleotides (synthetic, chemically modified and the like, as described for example in Applications EP 092 574, EP 231 495, WO 92/03568; WO 91/13080 and the like) or DNA sequences encoding RNAs capable of selectively interacting with the p53 mRNA, according to the technique described for example in application EP 140 308.

Such sequences may also be directed against the gene encoding p53 and act on its transcription into RNA. More particularly, these sequences may be directed against coding regions of the gene (p53 structural gene), or against noncoding regions: regions regulating transcription, exons and the like. Such sequences can be prepared under the conditions described for example in EP 558 634, WO 91/06626, WO 92/10590, WO 93/10820 and the like).

Among the compounds which act on the binding of p53 to DNA, there may be mentioned more particularly p53 antagonists, or proteins capable of interacting with p53 and of thus modulating its DNA-binding activity. In this regard, there may be mentioned the negative dominant mutants of p53 consisting essentially of inactive mutated form, which are capable of entering into competition with the wild-type protein for the interaction with DNA. Such mutants are for example the p53Val135 mutant, or other forms described for example in Michalovitz et al. [J. Cell. Bioch. 45 (1991) 22]. They can be used as such, but, preferably, they are

used within the framework of the present invention in the form of genetic constructs capable of expressing these mutants in vivo. Other compounds capable of at least partially inhibiting the binding of p53 to DNA consist of double-stranded nucleic acids reproducing the site for binding of p53 to DNA [El-Deiry et al., Nature 1 (1992) 45; Kern et al., Science 252, 1708; Friedman et al., PNAS 90 (1993) 3319]. The Applicant has indeed shown that such nucleic acids were capable of complexing the transcription factors present in the cells, of preventing them from attaching to their endogenous sites, and thus, of blocking their transcriptional activity.

In a preferred mode, the compound used within the framework of the present invention is a double-stranded nucleic acid comprising all or part of the site for the binding of p53 to DNA. More preferably, the nucleic acid comprises all or part of the sequence SEQ ID No. 2 or an active variant thereof. The term active variant designates, for the purposes of the invention, any variant of the sequence SEQ ID No. 2 which has conserved the properties of attachment to the p53 protein. Such variants can be obtained by mutation, deletion, substitution and/or addition of bases to the sequence SEQ ID No. 2, followed by verification in vitro of the binding activity.

In another preferred mode, the compound used within the framework of the present invention is a

nucleic acid encoding a mutated form of the p53 protein capable of antagonizing the activity thereof.

Still in a preferred mode, the compound used within the framework of the present invention is an antisense nucleic acid capable of reducing the levels of expression of the p53 protein, at the transcriptional or translational level. More preferably, it is a DNA encoding an antisense ribonucleic acid capable of inhibiting the translation of the p53 cellular mRNA. Such an antisense is represented on the sequence SEQ ID No. 1.

The nucleic acid can be used as such, for example after injection into man or animals, in order to induce a protection or to treat neuronal degeneration. In particular, it can be injected in naked DNA form according to the technique described in application WO 90/11092. It can also be administered in complexed form, for example with DEAE-dextran [Pagano et al., J.Virol. 1 (1967) 891], with nuclear proteins [Kaneda et al., Science 243 (1989) 375], with lipids [Felgner et al., PNAS 84 (1987) 7413], in the form of liposomes [Fraley et al., J.Biol.Chem. 255 (1980) 10431], and the like.

Preferably, the nucleic acid used within the framework of the invention forms part of a vector. The use of such a vector makes it possible, indeed, to enhance the administration of the nucleic acid into the cells to be treated, and also to increase its stability

in the said cells, which makes it possible to obtain a lasting inhibitory effect. Furthermore, it is possible to introduce several nucleic acid sequences into the same vector, which also increases the efficiency of the treatment.

The vector used may be of various origins, as long as it is capable of transforming animal cells, preferably human nerve cells. In a preferred embodiment of the invention, a viral vector is used which may be chosen from adenoviruses, retroviruses, adeno-associated viruses (AAV), the herpes virus, and the like.

In this regard, the subject of the present invention is also any recombinant virus comprising, inserted into its genome, a nucleic acid encoding a mutated form of the p53 protein capable of antagonizing the activity thereof, and/or a nucleic acid comprising all or part of the site for binding of p53 to DNA and/or an antisense nucleic acid capable of reducing the levels of expression of the p53 protein, at the transcriptional or translational level.

The recombinant virus according to the invention may be chosen from adenoviruses, retroviruses, adeno-associated viruses and the like. Preferably, it is a virus capable of infecting the nerve cells, such as especially an adenovirus. Vectors derived from adenoviruses, retroviruses or AAVs incorporating heterologous nucleic acid sequences have

been described in the literature [Akli et al., Nature Genetics 3 (1993) 224; Stratford-Perricaudet et al., Human Gene Therapy 1 (1990) 241; EP 185 573, Levrero et al., Gene 101 (1991) 195; Le Gal la Salle et al., Science 259 (1993) 988; Roemer and Friedmann, Eur. J. Biochem. 208 (1992) 211; Dobson et al., Neuron 5 (1990) 353; Chiocca et al., New Biol. 2 (1990) 739; Miyanohara et al., New Biol. 4 (1992) 238; WO91/18088].

Advantageously, the recombinant virus according to the invention is a defective virus. The term "defective virus" designates a virus incapable of replicating in the target cell. Generally, the genome of the defective viruses used within the framework of the present invention therefore lacks at least the sequences necessary for the replication of the said virus in the infected cell. These regions may be either removed (completely or in part), or made nonfunctional, or substituted by other sequences and especially by the nucleic acid. Preferably, the defective virus conserves nevertheless the sequences of its genome which are necessary for the encapsulation of the viral particles.

It is particularly advantageous to use the nucleic sequences of the invention in a form incorporated into a defective recombinant adenovirus.

There are, indeed, various adenovirus serotypes whose structure and properties vary somewhat, but which are not pathogenic for man, and especially non-immunodepressed subjects. Moreover, these viruses

do not integrate into the genome of the cells which they infect, and may incorporate large fragments of exogenous DNA. Among the various serotypes, the use of the type 2 or 5 adenoviruses (Ad 2 or Ad 5) is preferred within the framework of the present invention. In the case of Ad 5 adenoviruses, the sequences necessary for the replication are the E1A and E1B regions.

A specific embodiment of the invention consists in a vector, especially a viral vector, comprising at least two nucleic acids as defined above.

The defective recombinant viruses of the invention can be prepared by homologous recombination between a defective virus and a plasmid carrying, inter alia, the nucleic acid sequence as defined above [Levrero et al., Gene 101 (1991) 195; Graham, EMBO J. 3(12) (1984) 2917]. The homologous recombination occurs after co-transfection of the said viruses and plasmid into an appropriate cell line. The cell line used should preferably (i) be transformable by the said elements, and (ii) comprise the sequences capable of complementing the genome part of the defective virus, preferably in integrated form in order to avoid risks of recombination. By way of example of a line which can be used for the preparation of defective recombinant adenoviruses, there may be mentioned the human embryonic kidney line 293 [Graham et al., J. Gen. Virol. 36 (1977) 59] which contains especially,

integrated into its genome, the left-hand part of the genome of an Ad5 adenovirus (12 %). By way of example of a line which can be used for the preparation of defective recombinant retroviruses, the CRIP line may be mentioned [Danos and Mulligan, PNAS 85 (1988) 6460].

Next, the viruses which have multiplied are recovered and purified according to conventional molecular biological techniques.

The subject of the present invention is also a pharmaceutical composition comprising at least one recombinant virus as defined above.

The pharmaceutical compositions of the invention can be formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

Preferably, the pharmaceutical compositions contain pharmaceutically acceptable vehicles for an injectable formulation. They may be in particular saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like, mixtures of such salts), sterile solutions, isotonic solutions or dry compositions, especially freeze-dried compositions, which, upon addition, depending on the case, of sterilized water or of physiological saline, allow the constitution of injectable solutions.

The doses of nucleic acids (sequence or

vector) used for the administration can be adjusted according to various parameters, and especially according to the mode of administration used, the relevant pathology, the nucleic acid to be expressed, or alternatively the desired duration of the treatment. In general, as regards the recombinant viruses according to the invention, these are formulated and administered in the form of doses of between 10^4 and 10^{14} pfu/ml, and preferably 10^6 to 10^{10} pfu/ml. The term pfu ("plaque forming unit") corresponds to the infectious power of a virus solution, and is determined by infecting an appropriate cell culture, and measuring, generally after 48 hours, the number of plaques of infected cells. The techniques for the determination of the pfu titre of a viral solution are well documented in the literature.

Such pharmaceutical compositions can be used in man, for the treatment and/or prevention of neurodegenerative diseases, and in particular for the treatment and/or the prevention of neuronal degeneration associated with ischaemia, hypoxia, anoxia, hypoglycaemia, epileptic fits or alternatively cerebral and spinal traumas, or for the treatment and/or the prevention of Huntington's chorea, Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis.

The present invention will be described more fully with the aid of the following examples which

should be considered as illustrative and nonlimiting.

Legend to the figures

Figure 1 : Inhibition of cell death induced by glutamate on primary cultures of cortical neurones by an anti-p53 antisense nucleic acid.

General cloning techniques

The methods conventionally used in molecular biology, such as preparative extractions of plasmid DNA, centrifugation of plasmid DNA in caesium chloride gradient, agarose or acrylamide gel electrophoresis, purification of DNA fragments by electroelution, phenol or phenol-chloroform extraction of proteins, ethanol or isopropanol precipitation of DNA in saline medium, transformation in Escherichia coli and the like, are well known to persons skilled in the art and are widely described in the literature [Maniatis T. et al., "Molecular Cloning, a Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982; Ausubel F.M. et al. (eds), "Current Protocols in Molecular Biology", John Wiley & Sons, New York, 1987].

The pBR322- and pUC-type plasmids and the phages of the M13 series are of commercial origin (Bethesda Research Laboratories).

For the ligations, the DNA fragments can be separated according to their size by agarose or acrylamide gel electrophoresis, extracted with phenol or with a phenol/chloroform mixture, precipitated with ethanol and then incubated in the presence of phage T4

DNA ligase (Biolabs) according to the recommendations of the supplier.

The filling of the protruding 5′ ends can be performed with the Klenow fragment of E. coli DNA polymerase I (Biolabs) according to the specifications of the supplier. The destruction of the protruding 3′ ends is performed in the presence of phage T4 DNA polymerase (Biolabs) used according to the recommendations of the manufacturer. The destruction of the protruding 5′ ends is performed by a controlled treatment with S1 nuclease.

Site-directed mutagenesis in vitro by synthetic oligodeoxynucleotides can be performed according to the method developed by Taylor et al. [Nucleic Acids Res. 13 (1985) 8749-8764] using the kit distributed by Amersham.

The enzymatic amplification of the DNA fragments by the so-called PCR technique [Polymerase-catalyzed Chain Reaction, Saiki R.K. et al., Science 230 (1985) 1350-1354; Mullis K.B. and Faloona F.A., Meth. Enzym. 155 (1987) 335-350] can be performed using a DNA thermal cycler (Perkin Elmer Cetus) according to the specifications of the manufacturer.

The verification of the nucleotide sequences can be performed by the method developed by Sanger et al. [Proc. Natl. Acad. Sci. USA, 74 (1977) 5463-5467] using the kit distributed by Amersham.

EXAMPLES

Example 1 : Reduction in the volume of infarct in mice made ischaemic by suppression of the p53 gene.

This example describes the effect of the suppression of the p53 gene on the volume of infarct in mice made ischaemic. For that, ischaemias were induced in mice by occlusion of the middle cerebral artery, and the volumes of infarct were determined and then compared.

Procedure : The animals (C57/Blc male mice 9 to 12 weeks old, Genpharm, Denmark; wild-type homozygots or Δp53) were anaesthetized in a mixture of oxygen, nitrous oxide and 1.8 % halothane, and maintained under these conditions during the entire surgical procedure. The rectal temperature is maintained at 37°C +/- 0.5 by a heating cover. The left middle cerebral artery was then cauterized by electrocoagulation by means of a bipolar clip. The wound was then stitched up and the animals placed in a room at 30°C for 24 hours, with food and drink being available ad libitum. After 24 hours, the animals were sacrificed by decapitation. The brains were removed, immersed in an isopentane bath at -30°C and then stored at -80°C. 40 μm histological sections were then made in a cryostat at -20°C at the rate of one section every 500 μm, from the appearance of the infarction until it disappeared. These sections were then stained with Cresyl violet. The volume of the

infarction is determined by image analysis. The statistical analysis is performed by means of Student's t test for independent groups, after verification of the homogeneity of the various values. In the case where the various values were not homogeneous, the Wilcoxon's nonparametric test was used.

Results : The results obtained are presented in the table below.

	P53 mice	Control mice
Total number of animals tested	45	46
Mean weight (g)	25.73	25.44
Mean temperature (°C)	36.80	37.01
Mean volume of infarct (mm^3)	24.95+/-1.81	31.54+/-1.86

These results show a reduction of the order of 20 % in the volumes of infarct after ischaemia in the mice not expressing the p53 gene. These results therefore demonstrate that a suppression of p53 activity makes it possible to reduce neuronal degeneration.

Example 2 : Inhibition of cell death induced by glutamate of primary cultures of cortical neurones by an anti-p53 antisense nucleic acid

This example describes the effect of an anti-p53 antisense nucleic acid on death induced by

glutamate on embryonic rat cortical neurones, in primary culture.

Glutamate is the principal neurotransmitter exciting the central nervous system. However, exposure to glutamate for abnormally long periods, or to concentrations higher than the physiological concentrations can cause a neuronal toxicity designated by the term excitotoxicity [Olney Adv. Exp. Med. Biol. 203 (1986) 631]. Numerous experimental arguments suggest that this type of toxicity contributes to the neuronal degeneration associated with ischaemia, hypoxia, hypoglycaemia, epileptic fits or alternatively to cerebral traumas [Choi, J. Neurobiol. 23 (1992) 1261]. The excitotoxicity is also thought to be involved in the pathogenesis of diseases such as Huntington's chorea [Young et al., Science 241 (1988) 981] and Alzheimer's disease [Koh et al., Brain Res. 533 (1990) 315; Mattson et al.; J. Neurosci. 12 (1992) 376]. This example shows that the toxic effect of glutamate is partly inhibited in the presence of an antisense nucleic acid capable of reducing the levels of expression of the p53 protein.

Preparation and sequence of the antisense nucleic acid : The antisense oligonucleotide was synthesized by means of an automatic nucleotide synthesizer (Maniatis). The sequence of the oligonucleotide is as follows: 5'-CGACTGTGAATCCTCCAT-3' (SEQ ID No. 1).

Study of inhibition : Embryonic Wistar rat cortex cells (E17) were isolated according to the method of Dichter [Brain Res. 149 (1978) 279], cultured in 6-well Costar plates (35 min; density 6×10^5 cells/plate), in DMEM medium (Dulbecco's Modified Eagle Medium) containing 10 µg/ml insulin, 10 µg/ml transferrin, 10 ng/ml sodium selenite, 10 nM progesterone, 1 nM triiodothyronine, and stored in an oven (37°C, 5 % CO2). 2 µM anti-p53 antisense nucleic acid described above were then added to the cultures, during the inoculation, and then on days 1 and 2. The glutamate (5 mM) was administered on day 2, at the same time as the anti-p53 antisense nucleic acid. The toxicity induced by the glutamate was determined after 24 hours of culture, by measuring the mitochondrial activity according to the technique described by Manthorpe et al. [Dev. Brain. Res. 25 (1986) 191].

The results obtained are presented in Figure 1. They show clearly that the anti-p53 antisense nucleic acid is capable of reducing by about 25 % the toxicity induced by glutamate.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: RHONE-POULENC RORER S.A.

(B) STREET: 20, avenue Raymond ARON

(C) CITY: ANTONY

(D) COUNTRY: FRANCE

(E) POSTAL CODE: 92165

(ii) TITLE OF INVENTION: Pharmaceutical compositions and utilization thereof particularly for the treatment of neurodegenerative diseases.

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Tape

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENTIAL CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CGACTGTGAA TCCTCCAT

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENTIAL CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTISENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGACATGCCC GGGCATGTCC

CLAIMS

1. Use of a compound which at least partially inhibits the activity of the p53 protein for the preparation of a pharmaceutical composition intended for the treatment and/or the prevention of neurodegenerative diseases.

2. Use according to Claim 1, characterized in that it is a compound which acts on the synthesis of the p53 protein, at the transcriptional, translational or post-translational levels, and/or on the binding of p53 to DNA.

3. Use according to Claim 2, characterized in that the compound is a double-stranded nucleic acid comprising all or part of the site for binding of p53 to DNA.

4. Use according to Claim 2, characterized in that the compound is a nucleic acid encoding a mutated form of the p53 protein capable of antagonizing the activity thereof.

5. Use according to Claim 2, characterized in that the compound is an antisense nucleic acid capable of reducing the levels of expression of the p53 protein, at the transcriptional or translational level.

6. Use according to Claim 5, characterized in that the antisense nucleic acid is a DNA encoding an antisense ribonucleic acid capable of inhibiting the translation of the p53 cellular mRNA.

7. Use according to Claims 3 to 5,

characterized in that the nucleic acid forms part of a vector.

8. Use according to Claim 7, characterized in that the nucleic acid forms part of a viral vector.

9. Recombinant virus comprising, inserted into its genome, at least one nucleic acid encoding a mutated form of the p53 protein capable of antagonizing the activity thereof, and/or a nucleic acid comprising all or part of the site for binding of p53 to DNA and/or an antisense nucleic acid capable of reducing the levels of expression of the p53 protein, at the transcriptional or translational level.

10. Recombinant virus according to Claim 9, characterized in that it is an adenovirus, a retrovirus, an adeno-associated virus, or the herpes virus.

11. Recombinant virus according to Claim 10, characterized in that it is an adenovirus.

12. Recombinant virus according to one of Claims 7 to 10, characterized in that the nucleic acid comprises all or part of the sequence SEQ ID No. 2 or active variants thereof.

13. Recombinant virus according to one of Claims 9 to 12, characterized in that it comprises several identical or different nucleic acids as defined in Claims 3 to 5.

14. Recombinant virus according to one of Claims 9 to 13, characterized in that it is a defective

virus.

15. Pharmaceutical composition comprising at least one recombinant virus according to one of Claims 9 to 14.

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, by entry into the U.S. national stage of examination, on the invention entitled

PHARMACEUTICAL COMPOSITIONS AND UTILIZATION THEREOF PARTICULARLY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

the international specification of which was filed on September 29, 1994 as Application Serial No. PCT/FR94/01142, which notice of transmission was given on April 13, 1995, by the International Bureau. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of a foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications(s)			Priority Claimed	
FR93-11774	FR	4 October 1993	X	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status-Patented, Pending or Abandoned)

(Application Serial No.) (Filing Date) (Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Martin F. Savitzky	Reg. No. 29,699
Paul R. Darkes	Reg. No. 33,862
James A. Nicholson	Reg. No. 25,478
Raymond S. Parker, III	Reg. No. 34,893
Ross J. Oehler	Reg. No. 33,270
Julie K. Smith	Reg. No. 38,619

Send Correspondence to:
Julie K. Smith
Rhône-Poulenc Rorer Inc.
500 Arcola Road; #3C43
P.O. Box 1200
Collegeville, PA 19426-0107

Direct Telephone Calls to: (610) 454-3839

MALLET, Jacques	French
First or Sole Inventor	Citizenship
18, rue Charcot	Same
Residence Address	Post Office Address
75013 PARIS	Same
City	City
FRANCE	Same
State (Zip) or Country	State (Zip) or Country
Date	Signature

==========

REVAH, Frédéric — French
Second Inventor — Citizenship

49, rue de Châtenay, Bât. Flandre 2 — Same
Residence Address — Post Office Address

92160 ANTONY — Same
City — City

FRANCE — Same
State (Zip) or Country — State (Zip) or Country

Date — Signature

==========

STUTZMANN, Jean-Marie — French
Third Inventor — Citizenship

9, rue De l'Arche — Same
Residence Address — Post Office Address

94440 VILLECRESNES — Same
City — City

FRANCE — Same
State (Zip) or Country — State (Zip) or Country

Date — Signature

==========

Fourth Inventor — Citizenship

Residence Address — Post Office Address

City — City

State (Zip) or Country — State (Zip) or Country

Date — Signature

PATENT
Attorney Docket No. 3804.0114-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Jacques MALLET *et al.*	)	
	)	
Serial No.: 08/624,469	)	Group Art Unit: 1632
	)	
Filed: May 10, 1996	)	Examiner: J. Schmuck
	)	
For: PHARMACEUTICAL COMPOSITIONS AND UTILIZATION THEREOF PARTICULARLY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES	)	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

ASSOCIATE POWER OF ATTORNEY

I hereby appoint Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387;

Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W. Edmondson, Reg. No. 33,871; Michael R. McGurk, Reg. No. 32,045; Joann M. Neth, Reg. No. 36,363; Gerson S. Panitch, Reg. No. 33,751; Cheri M. Taylor, Reg. No. 33,216; Charles E. Van Horn, Reg. No. 40,266; Linda A. Wadler, Reg. No. 33,218; Jeffrey A. Berkowitz, Reg. No. 36,743; Michael R. Kelly, Reg. No. 33,921; James B. Monroe, Reg. No. 33,971; and Steven P. O'Connor, Reg, No. 41,225 as associate attorneys to prosecute this application, and all continuations and divisions thereof, and to transact all business in the Patent and Trademark Office connected therewith.

Please send all future correspondence and Office Actions to the following address:

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
1300 I Street, N.W.
Washington, D.C. 20005-3315

Respectfully submitted,

By: [signature]
Ross J. Oehler
Reg. No. 33,270

Date: April 29, 1999